REMARKS

In response to the Office Action of October 12, 2006, claims 1, 5, 7 and 9-12 are hereby amended. Claims 1-12 were rejected under 35 U.S.C. § 112, second paragraph and claims 1, 2, 4, and 9-12 were rejected under 35 U.S.C. § 102(b); claims 1 and 3-12 were rejected under 35 U.S.C. § 103(a) and claims 5-8 were rejected under 35 U.S.C. §§ 102(b)/103(a). Each of these rejections is discussed below.

Rejections under 35 U.S.C. § 112, second paragraph

The Examiner has rejected claims 1-12 under 35 U.S.C. § 112, second paragraph "as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention." More specifically, the Examiner provides that the following phrases lack antecedent basis: "the amount" (claim 1); "the dissociation constant and concentration" (claims 5 and 7); "the saturation point" (claim 9); "the concentration, amount and K_d" (claim 10); "the nonspecific binding" (claim 11); and "the effective concentration" (claim 12). In response to this rejection, each of these claims have been amended to provide antecedent basis for each of the phrases noted by the Examiner. Applicant respectfully requests that this rejection now be withdrawn.

Rejection Under 35 U.S.C. § 102

The Court of Appeals for the Federal Circuit has stated that anticipation requires the presence in a single prior art reference of each and every element of the claimed invention. *Lindemann Maschinenfabrik GMBH v. American Hoist & Derrick Co.*, 730 F.2d 1452, 1458 (Fed. Cir. 1984); *Alco Standard Corp. v. Tennessee Valley Auth.*, 1 U.S.P.Q.2d 1337, 1341 (Fed. Cir. 1986). "There must be no difference between the claimed invention and the reference disclosure, as viewed by a person of ordinary skill in the field of the invention." *Scripps Clinic v. Genentech Inc.*, 18 U.S.P.Q.2d 1001, 1010 (Fed. Cir. 1991) (citations omitted). To anticipate a claim, a prior art reference must disclose every limitation of the claimed invention either expressly or inherently." *Atlas Powder Co. v. Ireco Inc.*, 190 F.3d. 1342, 1346 (Fed. Cir. (1999) (quoting *In re*

Schreiber, 128 F.3d 1473, 1477 (Fed. Cir. 1997)). In light of this standard each of the rejections raised by the Examiner is addressed below.

The Examiner has rejected claims 1, 2, 4 and 9-12 under 35 U.S.C. § 102 (b) as being anticipated by Graham *et al.* (U.S. Pat No. 4,743,452). The Examiner reasons that Graham *et al.* teach increasing the dynamic range of an immunoassay and forestalling the hook effect by addition of unlabeled ligand binding partners to a solution containing sample and labeled antibodies. Applicant respectfully traverses this rejection

Graham *et al.* teach a method for reducing the "hook effect" in antibody-based sandwich type immunoassays. As provided in the Specification, the hook effect results from the first and second binding partners in a sandwich based assay being bound to different analytes. As a result, fewer sandwich complexes are formed giving a false lower concentration of ligand than is actually present in the sample (Graham *et al.* col. 2, lines 3-12). Thus the hook effect is a phenomenon which is particular to sandwich-based assays. The immunoassays disclosed by Graham *et al.* comprise a first ligand binding partner which is immobilized on a solid support and then reacted --thereby potentially forming a sandwich complex-- with a second ligand binding partner in accordance with the presence or absence of the ligand/analyte to be detected. To reduce the hook effect either the first ligand binding partner or the second ligand binding partner or a combination thereof is added in excess. As taught and claimed, the method is specific for antibody-based sandwich type immunoassays for use in the quantification of a single analyte (ligand). The reference does not teach or suggest that the method can be extended to a sample containing a mixture of analytes, to a non-sandwich based assay or to a non-antibody based assay.

The present invention on the other hand is drawn to a method for simultaneously quantifying high and low abundance analytes that may be contained in a biological sample. (Specification, paragraph 1). As noted in the background section of the invention, prior to the filing of the instant application, "protein levels [were] measured individually with assays tailored to each analyte of interest." (Specification, paragraph 3). Thus, one objective of the instant invention as set forth in paragraph 5 of the Specification was "to provide a general method for adjusting the inherent quantification

range of a particular set of analytes to higher concentration regions, leaving the range of the remaining analytes the same and thereby permitting the simultaneous and accurate quantification of a plurality of analytes over a wide range of concentration values." The present invention provides for the first time a method for analyzing simultaneously a potentially complex mixture of analytes using a non-sandwich based assay.

Referring to the claims, independent claim 1 is drawn to a method for decreasing the amount of a first analyte in a biological fluid that is capable of binding to a first capture reagent immobilized on a solid support without decreasing the amount of a second analyte in said biological fluid. The method comprises contacting the biological fluid with the first capture reagent free in solution. As provided in the Specification, "the addition of a quantity of the first capture reagent free in solution quantitatively specifically titrates the amount of the first analyte captured in the assay, lowering saturating levels of the first analyte to quantifiable levels," without affecting the concentration other analytes present in the sample. (Specification, paragraph 6). Thus, claim 1 is drawn to a method for decreasing the concentration of one analyte in a sample comprised of a mixture of analytes using a non-sandwich based assay. In contrast, as noted above, Graham *et al.* teach a method which is individually tailored to the analyte of interest using a sandwich based assay. Thus, Applicant maintains that this reference neither teaches nor suggests the method of the instant invention as set forth in claim 1.

Independent claims 9, 10 and 12 have been amended to specify that the capture reagent is a nucleic acid ligand. As noted above, Graham *et al.* teach a method involving antibody based immunoassays. As such, Applicant maintains that the method of Graham *et al.* does not anticipate the methods of the claims 9, 10 and 12 of the instant invention, as amended.

Finally, independent claim 11 is drawn to a method for lowering the nonspecific binding of an analyte in a biological fluid to a non-cognate capture reagent immobilized on a solid support. As noted above, Graham *et al.* teach a method for quantifying a single analyte in a sample using a sandwich based immunoassay, involving two capture reagents, only one of which is immobilized on the solid support. As such, Graham *et al.* do not address the presence of non-cognate capture reagents nor the problems associated

with the nonspecific binding of analytes to these reagents. Applicant maintains therefore that Graham *et al.* do not teach or suggest either expressly or inherently the method of claim 11.

For the reasons discussed above, Applicant maintains that the Graham *et al.* reference does not anticipate the methods of claims 1,2, 4 and 9-12, as amended, and therefore respectfully requests that this rejection be withdrawn.

The Examiner has rejected claims 1, 2, 4 and 9-12 under 35 U.S.C. § 102(b) as being anticipated by Neumann *et al.* (U.S. Pat No. 6,184,042). The Examiner reasons that Neumann *et al.* teach extending the measuring range of an immunoassay and reducing the hook effect by addition of oligomeric labeled ligand binding partners to a solution containing sample. Applicant respectfully traverses this rejection.

Neumann *et al.* teach a method for reducing the "hook effect" in antibody-based sandwich type immunoassays. As noted above, the hook effect results when the first and second binding partners in a sandwich based assay bind to different analyte and as such is a phenomenon which is particular to sandwich-based assays. As in the case of Graham *et al.*, the method is specific for antibody-based sandwich type immunoassays for use in quantification of a single analyte (ligand). The reference does not teach or suggest that the method can be extended to a sample containing a mixture of analytes, to a non-sandwich based assay or to a non-antibody based assay. For the reasons discussed above with respect to the Graham *et al.* reference, Applicant maintains that this reference does not teach or suggest the method of claims 1, 2, 4 and 9-12 of the instant invention as amended. Applicant therefore respectfully requests that this rejection be withdrawn.

The Examiner has rejected claims 1, 2, 4 and 9-12 under 35 U.S.C. § 102(b) as being anticipated by Piasio *al.* (U.S. Pat No. 4,098,876). The Examiner provides that Piasio *et al.* incubated sample with antibodies in solution before contacting the mixture with immobilized antibodies. Applicant respectfully traverses this rejection.

Piasio *et al.* teach a novel method for performing an antibody based sandwich type immunoassay. The method is comprised of a first incubation with the sample and a labeled antibody to form a first labeled immunochemical complex; followed by a second incubation between the complex formed in step (1) with an immobilized antibody, to

form a second labeled complex between the immobilized antibody and the first complex. The authors provide that "[b]y reversing the sequence of incubation steps in a 'two-site' or sandwich assay, greater sensitivity is achieved and an intermediate washing step is eliminated." (Abstract). Thus, Piasio *et al.* teach an improved method for performing an antibody based sandwich assay. As taught and claimed, the method is specific for sandwich type antibody-based immunoassays for use in quantification of a single analyte (ligand). The reference does not teach or suggest the method can be extended to a sample containing a mixture of analytes, to a non-sandwich based assay or to a non-antibody based assay.

Thus, for the reasons discussed above with respect to the Graham *et al.* reference and the Piasio *et al.* reference, Applicant maintains that this reference does not teach or suggest the method of claims 1, 2, 4 and 9-12 of the instant invention as amended. Applicant therefore respectfully requests that this rejection be withdrawn.

Rejections under 35 U.S.C. § 103(a)

The Examiner has rejected claims 1 and 3-12 under 35 U.S.C. § 103(a) as being unpatentable over Lin *et al.* (US 2002/0037506) in view of Graham *et al.* (U.S. Pat No. 4,743,452) or Neumann *et al.* ((U.S. Pat No. 6,184,042). The Examiner reasons that Lin *et al.* teach that sandwich type assays using aptamers are, like antibody-based assays, subject to the hook effect. However, Examiner acknowledges that the Lin *et al.* reference does not teach or suggest methods to reduce the hook effect in aptamer based assays. The Examiner further reasons that Graham *et al* and Neumann *et al* teach methods for reducing the hook effect in antibody-based assays. From this the Examiner concludes that it would have been obvious to combine the teachings of these references and that in doing so one would have a reasonable expectation that the known methods would successfully reduce the hook effect and increase the measuring range of the assay regardless of the nature of the ligand binding partner. Applicant respectfully traverses this rejection.

The Examiner bears the burden of establishing a prima facie case of obviousness. In determining obviousness, one must focus on Applicant's invention as a whole. *Symbol*

Technologies Inc. v. Opticon Inc., 19 USPQ2d 1241, 1246 (Fed. Cir. 1991). The primary inquiry is:

> whether the prior art would have suggested to one of ordinary skill in the art that this process should be carried out and would have had a reasonable likelihood of success. . . . Both the suggestion and the expectation of success must be found in the prior art, not in the applicant's disclosure.

In re Dow Chemical, 5 USPQ2d 1529, 1531 (Fed. Cir. 1988). In light of this standard each of the rejections raised by the Examiner is addressed below.

As noted above, the methods taught by Graham *et al.* and Neumann *et al.* are specific for antibody-based sandwich type immunoassays for use in quantification of a single analyte (ligand). The references do not teach or suggest that the methods can be extended to a sample containing a mixture of analytes, to a non-sandwich based assay or to a non-antibody based assay. Lin *et al.* teach an aptamer based two-site binding sandwich assay employing nucleic acid ligands rather than antibodies as the capture agents. Thus, like Graham *et al.* and Neumann *et al.* Lin teach a sandwich type assay. As noted by the Examiner, Lin *et al.* provide that similar to antibody-based sandwich assays, aptamer based assays also exhibit a "hook effect" at high target concentrations (page 3, paragraph 16), presumably for the same reasons. Also as noted by the Examiner, Lin *et al.* do not teach or suggest a method for reducing the "hook effect."

As noted above, the present invention is drawn to a method for simultaneously quantifying high and low abundance analytes that may be contained in a biological sample. Independent claim 1 is drawn to a method for decreasing the amount of a first analyte in a biological fluid that is capable of binding to a first capture reagent immobilized on a solid support without decreasing the amount of a second analyte in said biological fluid. The method comprises contacting the biological fluid with the first capture reagent free in solution. As provided in the Specification, "the addition of a quantity of the first capture reagent free in solution quantitatively specifically titrates the amount of the first analyte captured in the assay, lowering saturating levels of the first analyte to quantifiable levels," without affecting the concentration other analytes present in the sample. (Specification, paragraph 6). Thus, claim 1 is drawn to a method for

decreasing the concentration of one analyte in a sample comprised of a mixture of analytes using a non-sandwich based assay.

Independent claims 9, 10 and 12 are also drawn to methods which rely upon the addition of a quantity of the nucleic acid ligand capture reagent free in solution to quantitatively specifically titrate the amount of the analyte captured in the assay, thereby lowering saturating levels of the analyte to quantifiable levels.

Lin *et al.* on the other hand teach a ligand-based sandwich assay which is subject to the hook effect, but as noted by the Examiner do not teach or suggest a method for reducing this effect in a nucleic acid ligand based sandwich assay. The Examiner maintains, however, that the antibody-based methods of Graham *et al.* and Neumann *et al.* could obviously be extended to non-sandwich based assays involving nucleic acid ligands. Applicant respectfully disagrees.

An oligonucleotide ligand is a non-naturally occurring nucleic acid sequence having a specific binding affinity for a target molecule. The target binding activity of an oligonucleotide ligand is mediated by the direct interaction of nucleic acid residues with the target. This is the crucially important aspect of the function of the oligonucleotide ligand: they rely on non-naturally occurring interactions between nucleic acids and targets. An oligonucleotide ligand must adopt a specific three-dimensional structure in order to bind to its target. Oligonucleotide ligands have a variety of structural motifs, including hairpin loops, and symmetric and asymmetric bulges. These structures are specifically selected for during the SELEX process. Even minor perturbations to the structure of an oligonucleotide ligand can dramatically alter the ability of the oligonucleotide ligand to bind to its cognate target. Hence, one skilled in the art would recognize that the immobilization of an oligonucleotide ligand on a solid support would likely perturb the structure of that oligonucleotide ligand. Hence, one skilled in the art would have no reason to expect that methods applicable to antibodies could obviously be extended to methods involving oligonucleotide ligands. Nor does one skilled in the art have any reason to expect that methods applicable to sandwich assays could obviously be extended to the non-sandwich type assays of the instant invention. As provided in the Specification, "the addition of a quantity of the first capture reagent free in solution

quantitatively specifically titrates the amount of the first analyte captured in the assay, lowering saturating levels of the first analyte to quantifiable levels" without affecting the concentration other analytes present in the sample. None of the references relied upon by the Examiner taken together or alone teach or suggest that this is possible. As such, Applicant maintains that this combination of references does not render the method as set forth in claims 1, 9, 10 and 12 of the instant invention obvious.

Independent claim 11 is drawn to a method for lowering the nonspecific binding of an analyte in a biological fluid to a non-cognate capture reagent immobilized on a solid support. Both Graham *et al.* and Neumann *et al.* teach methods for quantifying a single analyte in a sample using a sandwich based immunoassay, involving two capture reagents, only one of which is immobilized on the solid support. As such, neither of these references address the presence of non-cognate capture reagents nor the problems associated with the nonspecific binding of analytes to these reagents. Although, Lin *et al.* do teach a method for quantifying multiple analytes, they likewise do not address the issue of non-specific binding to non-cognate capture reagents. Applicant maintains therefore that this combination of references do not teach or suggest either expressly or inherently the method of claim 11.

Applicant therefore respectfully requests that the Examiner reconsider this rejection.

Rejections under 35 U.S.C. § 102(b)/103(a)

The Examiner has rejected claims 5-8 under 35 U.S.C. § 102(b) as being anticipated by, or in the alternative under 35 U.S.C. § 103(a) as being obvious over Graham *et al.* (U.S. Pat No. 4,743,452) or Neumann *et al.* (U.S. Pat No. 6,184,042) or Piasio *et al.* (U.S. Pat No. 4,098,876). The Examiner reasons that it would have been implicit to have adjusted the concentration of the ligand binding partner in solution to the ranges as claimed to provide a usable standard curve. Applicant respectfully traverses this rejection.

As discussed above in detail each of the references relied upon by the Examiner teach a method for performing antibody-based sandwich type immunoassays for use in

quantifying a single analyte in a sample. Claims 5-8 of the instant invention depend from claim 1, which as set forth above is drawn to a method for decreasing the amount of a first analyte in a biological fluid that is capable of binding to a first capture reagent immobilized on a solid support without decreasing the amount of a second analyte in said biological fluid. The method comprises contacting the biological fluid with the first capture reagent free in solution. As noted above, the addition of a specific amount of the first capture reagent free in solution quantitatively specifically titrates the amount of the first analyte captured in the assay thereby lowering saturating levels of the first analyte to quantifiable levels, without affecting the concentration other analytes present in the sample. Thus, claim 1 is drawn to a method for decreasing the concentration of one analyte in a sample comprised of a mixture of analytes using a non-sandwich based assay. In contrast, as noted above, each of the references relied upon by the Examiner teach a method which is individually tailored to the analyte of interest using a sandwich based assay. Thus, Applicant maintains that these references neither teach nor suggest the method of the instant invention as set forth in dependent claims 5-8. In light of this Applicant respectfully requests that this rejection be withdrawn.

If it would be helpful to obtain favorable consideration of this case, the Examiner is encouraged to call and discuss this case with the undersigned.

This constitutes a request for any needed extension of time and an authorization to charge all fees therefore to deposit account No. 19-5117 if not otherwise specifically requested. The undersigned hereby authorizes the charge of any fees created by the filing of this document or any deficiency of fees submitted herewith to be charged to deposit account No. 19-5117.

Respectfully submitted,

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